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Applied Soil Ecology 35 (2007) 35–45
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Enzyme activities as affected by soil properties and land use in a tropical watershed☆

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Received 16 December 2005; accepted 26 May 2006

Abstract

Enzyme activities play key roles in the biochemical functioning of soils, including soil organic matter formation and degradation, nutrient cycling, and decomposition of xenobiotics. Knowledge of enzyme activities can be used to describe changes in soil quality due to land use management and for understanding soil ecosystem functioning. In this study, we report the activities of the glycosidases (β-glucosidase, α-galactosidase, and β-glucosaminidase), acid phosphatase, and arylsulfatase, involved in C (C and N for β-glucosaminidase), P, and S cycling, respectively, as affected by soil order and land use within a watershed in north-central Puerto Rico (Caribbean). Representative surface soil (0–15 cm) samples were taken from 84.6% of the total land area (45,067 ha) of the watershed using a completely randomized design. The activity of α-galactosidase was greater in soils classified as Oxisols than in soils classified as Ultisols and Inceptisols, and it was not affected by land use. The activity of β-glucosidase was greater in Oxisols compared to the Inceptisols and Ultisols, and it showed this response according to land use: pasture -> forest > agriculture. The activity of β-glucosaminidase was higher in Oxisols than the other soil orders, and it was higher under pasture compared to forest and agriculture. Acid phosphatase and arylsulfatase activities were greater in Oxisols and Ultisols than in Inceptisols, and they decreased in this order due to land use: forest = pasture > agriculture. As a group, β-glucosaminidase, β-glucosidase, and acid phosphatase activities separated the sites under forest and pasture from those under agriculture in a three-dimensional plot. Thus, enzyme activities in Inceptisols under agriculture could be increased to levels comparable to other soil orders with conservative practices similar to those under pasture and secondary forest growth. Our findings demonstrate that within this watershed, acid and low fertility soils such as Oxisols and Ultisols have in general higher enzyme activities than less weathered tropical soils of the order Inceptisols, probably due to their higher organic matter content and finer texture; and that the activities of these enzymes respond to management with agricultural practices decreasing key soil biochemical reactions of soil functioning. Published by Elsevier B.V.

Keywords: Enzyme activities; Inceptisols; Oxisols; Ultisols; Soil quality; Land use

1. Introduction

Microorganisms are the main source of enzymes in soils (Tabatabai, 1994), and thus the composition of the soil microbial communities strongly affects the potential of a soil for enzyme-mediated substrate catalysis

☆ This research was supported in part by NASA-URC-TCESS grant and by USDA-ARS-UPR Summer Research Internship, Lubbock, TX.
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0929-1393/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.apsoil.2006.05.012

(Kandeler et al., 1996). Microorganisms are responsible for innumerable processes that occur in soils. Soil enzymes (intracellular and extracellular) are the mediators and catalysts of biochemical processes important in soil functioning such as nutrient mineralization and cycling, decomposition and formation of soil organic matter, and decomposition of xenobiotics (i.e., pesticides). Specifically, the assessment of the activities of hydrolases can provide information on the status of key reactions that participate in rate limiting steps of the decomposition of organic matter and transformation of nutrients in soils. Thus, knowledge of several soil enzyme activities can provide information on the soil degradation potential (Trásar-Cepeda et al., 2000).

The assessment of soil enzyme activities is simple, requires low labor costs compared to other biochemical analysis (Ndiaye et al., 2000), and the results are correlated to other soil properties (Klose et al., 1999; Moore et al., 2000; Ndiaye et al., 2000; Trásar-Cepeda et al., 2000). Further, it has been reported that any change in soil management and land use is reflected in the soil enzyme activities, and that they can anticipate changes in soil quality before they are detected by other soil analyses (Ndiaye et al., 2000). Previous studies with soils from various regions have shown that enzyme activities are sensitive to soil changes due to tillage (Kandeler et al., 1999; Acosta-Martínez and Tabatabai, 2001), cropping systems (Bandick and Dick, 1999; Klose et al., 1999; Ndiaye et al., 2000; Ekenler and Tabatabai, 2002), and land use (Staben et al., 1997; Gewin et al., 1999; Acosta-Martínez et al., 2003b).

Most of the studies on soil enzyme activities have focused on temperate regions. There is scant information on enzyme activities in the tropics (Chander et al., 1997; Cleveland et al., 2003) and we are unaware of information available about the enzyme activities of soils in Puerto Rico (Caribbean island). This study investigated enzyme activities known to play critical roles in organic matter decomposition and mineralization of C, N, P, and S nutrients in soils, and that are reported to be sensitive to changes in soil quality due to different management and land uses in soils from humid and semiarid regions (Bandick and Dick, 1999; Ndiaye et al., 2000; Acosta-Martínez et al., 2003a, 2003b, 2004a). For example, the glycosidases are a group of C cycling enzymes that play a key role in the breakdown of low molecular weight carbohydrates by producing sugars; the main source of energy to soil microorganisms. β-Glucosidase activity, the most predominant glycosidase in soil, was studied because it is involved in the last limiting step of cellulose degradation. Another glycosidase, α-galactosidase, also named melibiase, was studied because it catalyzes the hydrolysis of the disaccharides, α-D-galatopyranosides, in soils. β-Glucosaminidase activity is another glycosidase studied because no information is available about chitin degradation in soils from tropical environments. β-Glucosaminidase is a key enzyme involved in the hydrolysis of *N*-acetyl-β-D-glucosamine residues from the terminal non-reducing ends of chitooligosaccharides (Parham and Deng, 2000). This hydrolysis is considered to be important in C and N cycling in soils because it participates in the processes whereby chitin is converted to amino sugars, a major source of easily mineralizable C and N in humid soils (Stevenson, 1994; Ekenler and Tabatabai, 2002). β-Glucosaminidase activity has been positively correlated with the cumulative N mineralized in soils (Dodor and Tabatabai, 2002; Ekenler and Tabatabai, 2002), microbial biomass C and N, and with fungi populations, as indicated by fungal indicator fatty acids (i.e., 18:2ω6c) (Parham and Deng, 2000; Acosta-Martínez et al., 2004b). In addition, acid phosphatase activity was studied because it catalyzes the hydrolysis of a variety of organic and inorganic phosphomonoesters, and is therefore, important in soil P mineralization and plant nutrition. The phosphatases are significantly affected by soil pH, which controls phosphorus availability in soil, and this could occur despite the level of organic matter content or disturbance. Arylsulfatase activity was studied because it is generally used to investigate organic S mineralization in soils.

Most of the studies that have evaluated enzyme activities in temperate areas have been performed under replicated controlled field conditions, and studies done at the watershed scale are rare. There are studies under way that are evaluating the C content of soils (Beinroth et al., 2003; Cruz, 2004; Suárez-Rozo, 2005), above-ground forest diversity (Suárez-Rozo, 2005), and water quality (Sotomayor-Ramírez et al., 2004) within the Rio Grande de Arecibo (RGA) watershed of Puerto Rico, but there is no information on the microbial ecological functioning of the main watershed soil orders (Ultisols, Inceptisols, and Oxisols) as affected by agriculture, forest, and pasture. This watershed has ecological importance for Puerto Rico because it contains numerous natural reserves, serves as hydrologic recharge to an important aquifer system in northern Puerto Rico, and provides potable water to more than one million residents of metropolitan San Juan. Information of several enzyme activities in the soils from the RGA watershed of

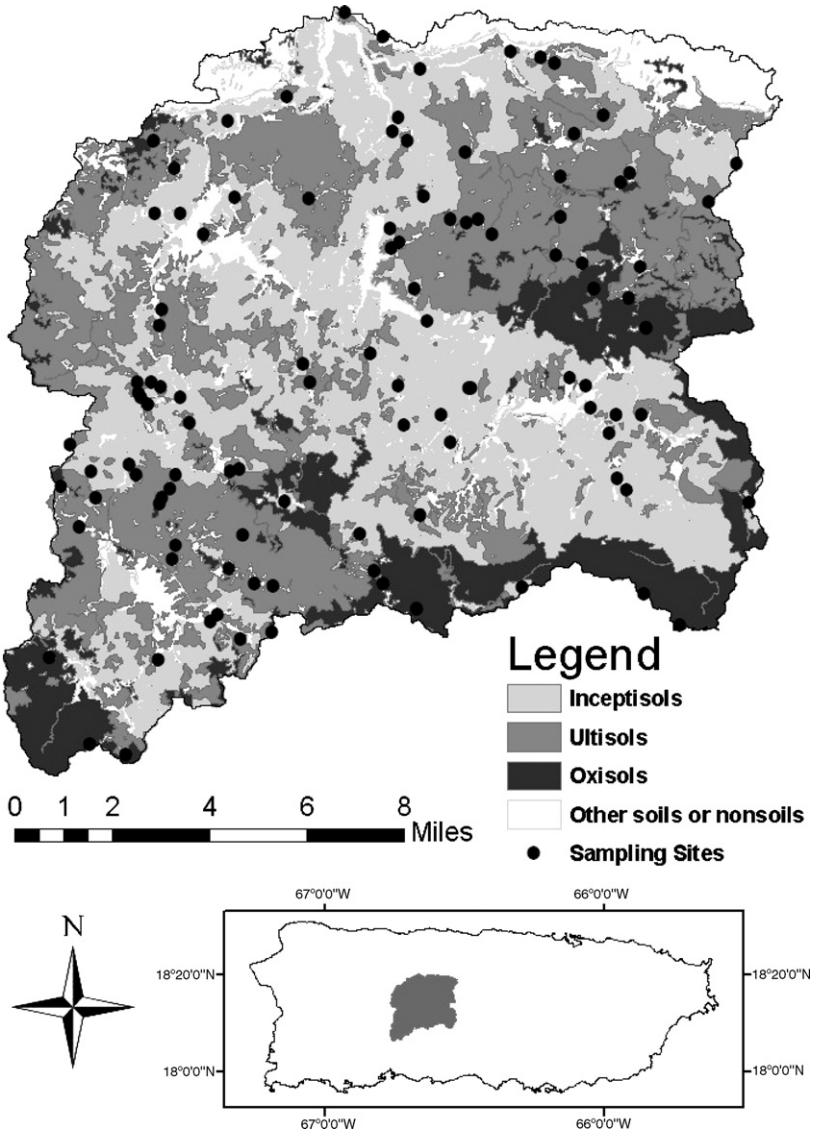

Fig. 1. The Rio Grande de Arecibo watershed is located in the north-central part of Puerto Rico.

Puerto Rico will assist to better understand the soils ecosystem functioning as affected by typical land use. A study of several enzyme activities within a hydrologic unit can provide information on the simultaneous influence of soils, climatic, and vegetative factors on soil functioning. Furthermore, understanding the functioning of soils within a watershed can be used to evaluate their influence on water quality, and ultimately provide information on the whole ecosystem environmental quality. Thus, the objective of this work was to provide information of selected enzyme activities involved in C, N, P, and S cycling in soils from the RGA watershed as affected by soil order and land use.

2. Materials and methods

2.1. Study site

The RGA watershed is located in the north-central part of Puerto Rico and is bordered by latitudes 18°11′N and 18°20′N to the north and south, respectively, and longitudes 66°32′W and 66°46′W to the east and west, respectively (Fig. 1). It has an area of 45,067 ha, which ends at Lago Dos Bocas (lake). The geology is dominated by cretaceous volcanic rocks and plutonic rocks, tertiary limestones, and quarternary alluvial deposits (Beinroth et al., 2003). There are 35 soil series which are subdivided into 79 mapping units with the

major soil orders being Ultisols, Oxisols, and Inceptisols (Acevedo, 1982; Gierbolini et al., 1979). The latest land use evaluation was performed in 2000 (PRASA, 2001), of which the total non-urban land area describes 32,006 ha (71.2%) as forest land, 5706 ha (12.6%) as water, rocky outcrops, and wetlands, 3776 ha (8.3%) as pasture land, and 3579 ha (7.9%) as agricultural land.

Further details on land use within the area and site description can be found in Cruz (2004) and (Suárez-Rozo, 2005). In brief, the agricultural sites studied were primarily under long-term semi-intensive coffee (*Coffea arabica*) production intermixed with an upper story of shade trees such as guaba (*Inga vera*), moca (*Andira inermes*) and guamá (*Inga* spp.); other crops include plantain (*Musa* spp.), yam (*Dioscorea* spp.) and citrus (*Citrus* spp.). The pasture sites generally have grass species such as: malojilla (*Eriochloa polytachya*), malojillo (*Urochloa mutica*), stargrass (*Cynodon nlemfuensis*), and pangola (*Digitaria eriantha*). The forest sites are secondary forests resulting from short-term use in agriculture and subsequent abandonment due to declining yields and poor management. The forest sites have areas stocked with deciduous, semi-deciduous or evergreen trees with crown closure percentage of 10% or more, capable of producing timber or other wood products, and exert an influence on climate and water regime (Suárez-Rozo, 2005). Among 81 species reported in a forest inventory, 25 of them are introduced, 6 are endemic and the rest (52) are native to Puerto Rico and Virgin islands (Suárez-Rozo, 2005). The dominant trees species in the forest sites are *Guarea guidonia*, *Cecropia schereberiana*, *Inga vera*, *Prestoea montana*, *Dendropanax arboreus*, *Didymopanax morototoni*, and *Syzygium jambos*.

2.2. Soil sampling

A geographic information system (GIS) was created that included watershed delineation based on USGS digital elevation model maps (DEM) (1:20,000 scale), soil mapping units (USDA-NRCS, 2001), primary and secondary roads (ESRI, 2000) and an IKONOS image of the area (Space Imaging, 2001). The soil mapping units and their respective polygons were identified using the GIS layers.

A total of 103 soil samples were taken at 0–15 cm depth from the RGA watershed (Table 1). Soil samples were taken from Oxisols, Ultisols, and Inceptisols under pasture, forest, and agriculture. Because the size of the experimental units (soil mapping units) varied in terms of number and area within the watershed, a stratified sampling approach was utilized. A total of 18 mapping units were selected for sampling, which represent the most extensive soils of the watershed. Each mapping unit has an area greater than 450 ha for a combined area of 33,322 ha (representing 84.6% of the total land area). Based on area distribution within a soil mapping unit, one sample was taken for approximately every 500 ha; thus some mapping units had more sampling points than others (Fig. 1). For example, most of Puerto Rico's landscape is young (geologically) with steep mountain slopes and recently deposited coastal sediments. Therefore, most soils on the island and in this watershed are classed as Inceptisols. Ultisols are next in predominance in the watershed. Oxisols are less prevalent than Ultisols and Inceptisols, and are generally located in geographic areas where extreme weathering has occurred which are in the steepest portions of hillslides. These areas were also the first to be abandoned for the implementation of conservation practices and forest development so that very small land areas dedicated to agriculture are present in the watershed.

Table 1
Number of soil samples collected from each soil order and land use studied within the Rio Grande de Arecibo watershed

Soil order	Land use			Total
	Agriculture	Forest	Pasture	
Inceptisols	14	24	6	44
Oxisols	2	5	1	8
Ultisols	11	27	13	51
Total	27	56	20	103

Based on the taxonomic classification of each mapping unit, the latter were then grouped by soil order for the statistical analyses. Due to the difficulty in accessing some areas, only mapping units (represented as a polygons in the GIS) that intersected primary (state or municipal) road were selected, and were assumed to be representative of all possible mapping units within the watershed. The geographic sampling points were selected by choosing random numbers from a list of available numbers corresponding to the kilometer numbers of the roads that coincided with each soil polygon. One soil sample corresponding to a mapping unit greater than 500 ha was obtained, with each mapping unit consisting of one or more polygons. Each polygon selected, had a specific soil taxonomic classification and had a particular land use associated with it. At each sampling point, the geographic coordinates were identified, and the land use was verified from the land use map (PRASA, 2001) and the space image. The pre-selected sampling site was

Table 2
Selected properties of the soils from the RGA watershed at 0–15 cm depth

Property	Inceptisols	Oxisols	Ultisols
Organic C (%)	1.61 (0.32, 4.68)[a]	3.82 (1.93, 6.42)	2.59 (1.21, 5.07)
Total N (%)	0.156 (0.03, 0.40)	0.316 (0.17, 0.64)	0.25 (0.02, 0.42)
C:N	10.18 (8.42, 13.9)	13.41 (8.11, 19.86)	10.41 (8.23, 15.58)
Soil pH (1:2.5 ratio)	5.3 (3.8, 6.6)	4.5 (3.7, 6.6)	4.9 (3.7, 7.1)
Clay (%)	32 (13, 63)	51 (38, 59)	46 (30, 66)
Silt (%)	29 (19, 45)	32 (20, 40)	32 (21, 47)
Sand (%)	39 (10, 68)	17 (12, 20)	22 (8, 45)

[a] Minimum and maximum values are in parentheses.

identified in the field by navigating with a global positioning system (Trimble Pro XR, Trimble Inc., Sunnyvale CA).

2.3. Soil properties

The organic C and N, texture, and pH of the soils are described in Table 2. Soil texture was determined using a laser diffraction particle size analyzer (Beckman-Coulter LS-230; Fullerton, CA). The determination of soil texture using the LS-230 has been positively and significantly correlated to the pipette method (Zobeck, 2004). In general, Inceptisols are coarser textured, and predominantly derived from plutonic rocks (Beinroth et al., 2003). Ultisols and Oxisols are finer textured, and are derived from volcanic rocks. Soil organic C and N were quantified in air-dried soil (<180 μm) using a LECO CN analyzer (Cruz, 2004). Soil pH was measured in a 1:2.5 (soil:water) mixture using a glass electrode.

The activities of β-glucosidase, α-galactosidase, arylsulfatase, and acid phosphatase were assayed using 1 g of air-dried soil (<2 mm) with their appropriate substrate and incubated for 1 h (37 °C) at their optimal pH as described in Tabatabai (1994). β-Glucosaminidase activity was determined similarly with the method of Parham and Deng (2000). A summary of the assay conditions, reactions, and role of these enzymes in soil function is provided in Table 3. The enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from a sample control value.

Table 3
The methods used for assay of soil enzyme activities in the systems studied

Class/EC number	Recommended name[a]	Role in soil function	Assay conditions[b]		
			Reaction	Substrate	Optimum pH
3.2.1.21	β-Glucosidase	Cellulose degradation, producing glucose needed by plants and microorganisms	Glucoside-R + H_2O → Glucose + R–OH	*p*-Nitrophenyl-β-D-glucopyranoside (10 mM)	6.0
3.2.1.21	α-Galactosidase	Melibiase degradation, producing glucose needed by plants and microorganisms	Glucoside-R + H_2O → Glucose + R–OH	*p*-Nitrophenyl-α-D-galactopyranoside (10 mM)	6.0
3.2.1.30	β-Glucosaminidase[c]	Chitin degradation, providing amino sugars, one of the major sources of mineralizable N	R-*N*-acetyl-β-D-glucosaminide → R–OH + *N*-acetyl-β-D-glucosaminide	*p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminidemine (10 mM)	5.5
3.1.3.2	Acid phosphatase	Produces plant available phosphates, predominant in acid soils	RNa_2PO_4 + H_2O → R–OH + Na_2HPO_4	*p*-Nitrophenyl phosphate (10 mM)	6.5
3.1.6.1	Arylsulfatase	Produces plant available sulfates	Phenol sulfate + H_2O → phenol + sulfate	*p*-Nitrophenyl sulfate (10 mM)	5.8

[a] Methods used are described in Tabatabai (1994).
[b] Values in parentheses are the substrate concentrations under assay conditions. Product of reaction is *p*-Nitrophenol = PN.
[c] Method used is described in Parham and Deng (2000).

Table 4
Statistical analysis for enzyme activities involved in C, N, P, and S cycling in soils from the RGA watershed at 0–15 cm depth

Source	β-Glucosidase	α-Galactosidase	β-Glucosaminidase	Acid phosphatase	Arylsulfatase
Land use (LU)	*	NS[a]	*	*	*
Order (O)	*	**	*	**	*
LU × O	NS	*	NS	*	NS

[a] No significance.
* $P < 0.1$.
** $P < 0.05$.

2.4. Statistical analyses

The statistical design was completely randomized with soil order and land use as main effects. Analyses of variance (ANOVA) and multivariate ANOVA (MANOVA) were performed using InfoStad program (version 1.1) to determine the significant effects of soil order and land use on the enzyme activities studied. Means separation was performed using Fisher's least significant difference (LSD) test at $P < 0.1$.

3. Results

The α-galactosidase activity in soils ranged from 1.16 to 40.7 mg product kg^{-1} soil h^{-1}, and it was significantly affected ($P < 0.05$) by soil order but not by land use (Table 4). For example, the activity of this enzyme was lower in the Inceptisols (4.14 mg product kg^{-1} soil h^{-1}) and Ultisols (4.84 mg product kg^{-1} soil h^{-1}) than in the Oxisols (7.31 mg product kg^{-1} soil h^{-1}) (Fig. 2).

The β-glucosaminidase activity ranged in the soils from 1.11 to 73.4 mg product kg^{-1} soil h^{-1}. The activity of this enzyme was significantly affected by soil order and land use (Table 4). Similar to α-galactosidase activity, lower β-glucosaminidase activity was found in the Inceptisols (13.24 mg product kg^{-1} soil h^{-1}) and Ultisols (15.0 mg product kg^{-1} soil h^{-1}) than in the Oxisols (19.25) (Fig. 2). The activity of this enzyme was lower in soils under agriculture and forest compared to soils under pasture (Fig. 3).

The β-glucosidase activity ranged in the soils from 1.04 to 63.4 mg product kg^{-1} soil h^{-1}. Similar to β-glucosaminidase activity, β-glucosidase activity was affected by land use and soil order (Table 4). Higher β-glucosidase activity was observed under Oxisols compared to Inceptisols (Fig. 2). The activity of this enzyme was greater in soils under pasture than under forest and agriculture (Fig. 3).

Acid phosphatase activity ranged in the soils from 40.27 to 498.6 mg product kg^{-1} soil h^{-1}. The activity of acid phosphatase was significantly affected by soil order and land use, and the interaction of these factors (Table 4). This enzyme activity showed this response: Inceptisols < Oxisols < Ultisols (Fig. 2). The activity of this enzyme showed this response due to land use: agriculture < forest = pasture (Fig. 3).

Arylsulfatase activity was significantly affected by soil order and land use (Table 4). This enzyme activity

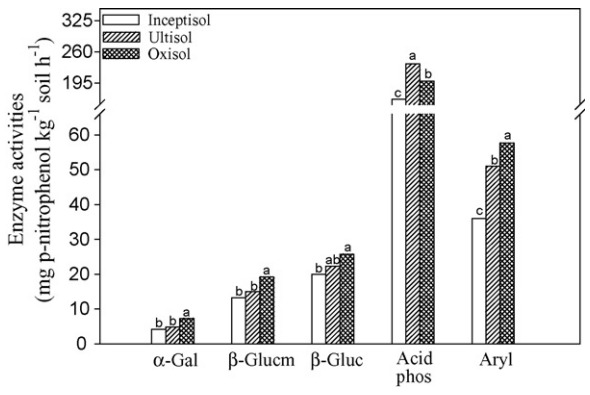

Fig. 2. Enzyme activities involved in C, N, P, and S cycling in soils (0–15 cm depth) from the RGA watershed as affected by soil order. For each enzyme activity, the least significant difference (LSD) according to $P < 0.1$ is given when differences are significant due to soil order.

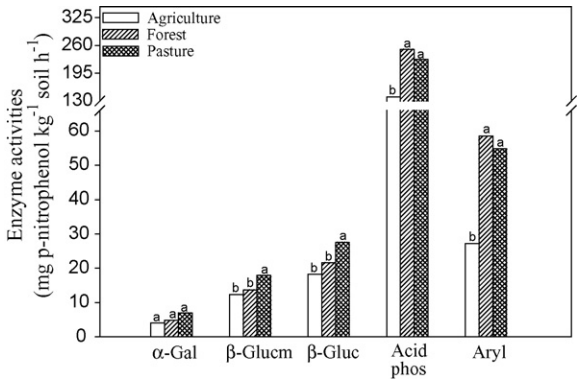

Fig. 3. Enzyme activities involved in C, N, P and S cycling from soils (0–15 cm depth) at RGA watershed as affected by land use. For each enzyme activity, the least significant difference (LSD) according to $P < 0.1$ is given when differences are significant due to land use.

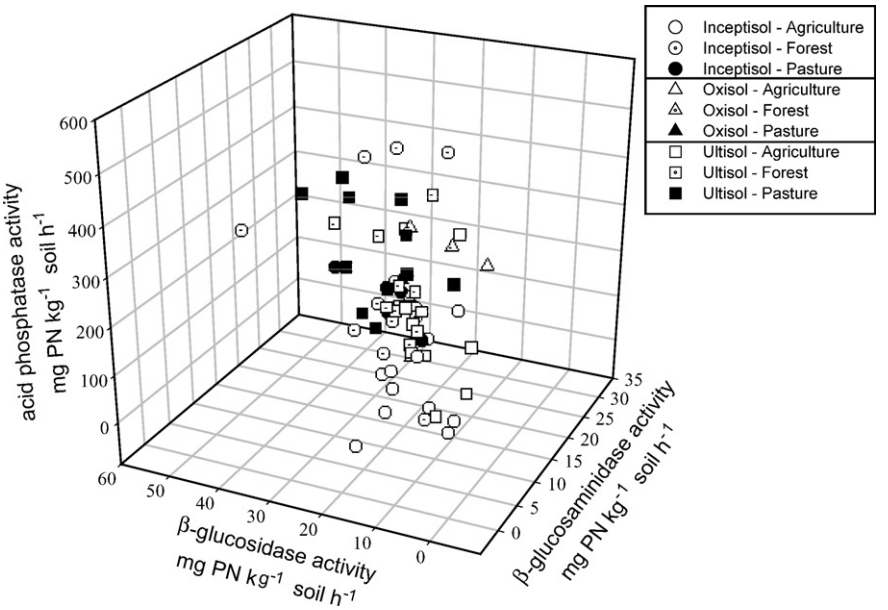

Fig. 4. Three-dimensional plot of the group of β-glucosidase, acid phosphatase, and β-glucosaminidase activities (0–15 cm depth) in soils from the RGA watershed as affected by soil order and land use.

showed this response due to soil order: Inceptisols < Ultisols < Oxisols (Fig. 2). Similar to acid phosphatase activity, the activity of this enzyme showed this response due to land use: agriculture < forest = pasture (Fig. 3).

A three-dimensional plot of β-glucosidase, β-glucosaminidase, and acid phosphatase activities together showed a separation of the Oxisols, Inceptisols, and Ultisols under agriculture from the rest of the soils under pasture and forest (Fig. 4). In addition, Inceptisols under pasture and forest clustered close to the Ultisols and Oxisols.

4. Discussion

4.1. Predominance of enzyme activities in soil

Among the enzymes studied, acid phosphatase and arylsulfatase activities were more predominant than the activity of the glycosidases β-glucosaminidase, β-glucosidase and α-galactosidase. Studies in semiarid soils (Acosta-Martínez et al., 2003a, 2003b) and humid soils (Klose et al., 1999; Klose and Tabatabai, 2002) have found that the glycosidases β-glucosaminidase and α-galactosidase are less predominant than other enzymes. The predominance of soil enzyme activities is more related to the ecological role and kinetic characteristics of the enzymes studied despite the effects of chemical and physical properties, geology, and land use of the soils studied (Tabatabai, 1994).

The values of β-glucosidase activity in the soils of the RGA watershed were lower than values reported for soils from humid temperate regions (Emmerling et al., 2002). The α-galactosidase activity in these soils was six to eight times lower than the values reported for a soil in Iowa, USA from a humid region (Acosta-Martínez and Tabatabai, 2000). Although there is little information of β-glucosaminidase activity in soils from diverse climatic regions, the values found for this enzyme activity were within the ranges of those reported for semiarid soils in Texas, USA (Acosta-Martínez et al., 2003a, 2003b). The values of arylsulfatase activity under pasture were about two times lower than those reported for high clay semiarid soils under pasture (Acosta-Martínez et al., 2003a, 2003b). These findings are presumably due to the fact that the microbial biomass, which is the principal source of enzymes in soils, is generally greater in soils from cooler, wetter regions than in soils from warmer, dryer regions (Allison, 1973; Spain et al., 1983). The greater microbial biomass results from the higher organic matter content and differences in organic matter quality between soils from cooler regions and soils from warmer regions. Generally, the degradation of organic matter exceeds the rate of humus synthesis in soils from warmer regions. In addition, the lower enzyme activities in the tropical region studied may result from differences in the type of clay minerals present in the soils from warmer regions. Clay minerals in cooler soils tend to be less weathered than those in soils from

warmer regions, which tend to be highly weathered (i.e., kaolinite, gibbsite, and hydrous Fe-oxides are commonly associated to Ultisols and Oxisols). Thus, we hypothesize that the lower enzyme activities found in these tropical soils compared to humid soils, may be related to lower expected microbial biomass, which was not quantified.

4.2. Enzyme activities as affected by land use and soil properties

The variation in enzyme activities as affected by the soil orders is associated in part with the geology of the parent material. Generally, higher enzyme activities were found in the Oxisols and Ultisols compared to the Inceptisols, which are young soils with only initial horizon development. Erosion rates are high because of the rugged mountain relief: therefore, new parent material is being exposed on the slopes. In addition, Inceptisols tend to be coarser textured, occur on eroded landscapes, and are predominantly derived from plutonic rocks in this watershed (Beinroth et al., 2003). In contrast, Ultisols and Oxisols are advanced weathering stage-soils, but are finer textured and are derived from volcanic rocks. In addition to the geology and soil texture, the variation of the enzyme activities is due to the differences in organic C content among soil orders. Cruz (2004) observed greater soil organic C content (0–15 cm) in Ultisols (5.00 kg C m^{-2}) and Oxisols (6.00 kg C m^{-2}) compared to Inceptisols (3.50 kg C m^{-2}).

The trends found in the enzyme activities as affected by land use are in agreement with previous studies showing lower values in cultivated soils when compared to the corresponding undisturbed or less-disturbed soils (Pascual et al., 1999; Acosta-Martínez et al., 2003b, 2004b). Our findings that soils under pasture sustained higher enzyme activities, compared to the corresponding agricultural soils, are due to the positive impacts of the surface cover, vegetation, and lack of tillage of pasture on soil properties including the microbial populations and activities. Previous studies have shown that changes in the microbial communities may influence the potential of soils for enzyme (i.e., hydrolases)-mediated substrate catalysis (Kandeler et al., 1996). A previous study found that the higher enzyme activities under pasture than soils under agriculture were correlated to higher microbial biomass and fatty acid indicators of protozoa (20:4ω6c) and fungal (18:3ω6c) populations (Acosta-Martínez et al., 2004b). McKinley et al. (2005) reported higher abundance of microbial phospholipids fatty acids (PLFA), indicative of higher microbial diversity, in soils under virgin prairie and long-term restoration sites compared to agricultural tilled soils.

A summary by Smith and Paul (1990) reported that generally soil microbial biomass C values in surface soil increase in the order: agriculture < forest < grassland. This is similar to our findings regarding some of the enzyme activities. The higher activities of the glycosidases (β-glucosidase and β-glucosaminidase) in pasture soils than in the forest soils at the RGA watershed may be attributed to grazing activities and the manure incorporation into the pasture soil. In addition, these findings are in agreement with previous studies showing that pasture or grassed areas can sequester more soil organic carbon than forest areas due to the large amounts of biomass and high rate of biomass turnover in pastures (Neill et al., 1997; Yakimenko, 1997). Acid phosphatase and arylsulfatase activities were similar under forest and pasture soils probably because both systems had acidic pH. Similarly, Cleveland et al. (2003) reported similar phosphatase activity under both forest and pasture for an Oxisol in Costa Rica, but they found higher microbial biomass under forest than in pasture.

In soils under agriculture, forest, and pasture of the RGA watershed, between 2 and 7% of the soil organic carbon (SOC) was quantified as labile C (Sotomayor-Ramírez et al., 2005). While we found differences in the enzyme activities due to land use, a recent study in the watershed did not find land use effects on labile or stable C concentrations, yet clayey Ultisols had higher labile C concentrations than coarser-textured Inceptisols (Sotomayor-Ramírez et al., 2005). Another study in the RGA watershed found differences in SOC content between forest and pasture land at 0–15 cm depth, and it was always higher compared to cropland in agreement with the trends of the enzyme activities (Cruz, 2004). The agricultural soils studied were mainly under long-term semi-intensive coffee (*Coffea* spp.) production, which are usually only cultivated during the initial planting, but are maintained weed free by mechanical or chemical management. Thus, C input to soils is reduced as compared to the corresponding less disturbed soils, which also explains the lower enzyme activities under agriculture compared to pasture. Interestingly, our findings showed no significant differences in the degradation of simple substrates such as chitin, cellulose, and melibiose in soils under agriculture (semi-intensive coffee production) and forest of the watershed, indicated by β-glucosaminidase, β-glucosidase, and α-galactosidase activities, respectively. These findings may suggest that a more mature tree

cover may be needed in the forest for detection of differences between forest and agriculture in terms of these enzyme activities. Puerto Rico's nearly 890,000 ha of land were mostly forested in the 16th century but by the late 1940s forest covered only 6% of the land area (Aide and Grau, 2004). Since then, the proportion of forest area has increased to about 34% island-wide and to nearly 72% in the RGA watershed because cropland and pasture were abandoned on eroded hillsides (Birdsey and Weaver, 1987). Thus, the forest land in the RGA watershed is primarily that resulting from the abandonment of cropland or pasture, and the regeneration of previous cutover or disturbed forest land. However, acid phosphatase and arylsulfatase activities, involved in P or S mineralization, were higher in forest than under agriculture. Our findings demonstrated the importance of evaluating enzyme activities for several different biochemical reactions in soils because their responses may vary depending on the land use history management.

Analysis of different enzyme activities together can provide a better picture of the status of soil processes and functioning (Acosta-Martínez et al., 2003b). A three-dimensional plot for the group of β-glucosidase, β-glucosaminidase, and acid phosphatase activities was useful to observe the interaction effect of soil order and land use on these three enzyme activities together for the RGA watershed. For example, most of the Ultisols and Inceptisols under agriculture showed separation (lower activities) from the rest of the systems in terms of these biochemical reactions as a group. This plot also showed that the forest and pasture land uses tended to increase these enzyme activities in the Inceptisols comparable to the other soil orders. These findings show the importance of conservative land uses such as forest and pasture to improve nutrient cycling and biochemical functioning of younger soils in the watershed, such as those of the Inceptisol order. Recent studies have suggested that the development and adoption of conservation and sound management practices (i.e., crop residues, manure or compost) will be needed for low organic matter soils of the watershed in order to increase soil C sequestration (Cruz, 2004). Previously, Martens et al. (2003) reported that the practice of permanent pastures and afforestation of agricultural land showed long-term potential for mitigation of atmospheric CO_2. In general, because the enzyme activities studied play important roles in key biochemical reactions of element transformation in soil, the trends found in this study support previous findings that cultivation in the watershed can result in loss of soil quality (Dick, 1997; Acosta-Martínez et al., 2003b).

Among the enzyme activities studied, the phosphatases, in addition to being affected by soil organic matter, are also influenced by soil pH. Previous studies have reported that phosphomonoesterases such as acid and alkaline phosphatases are significantly affected by changes in soil pH being more predominant in acidic and alkaline soils, respectively (Eivazi and Tabatabai, 1977). However, none of the enzyme activities were correlated to soil pH in the watershed. Other studies, involving diverse soil types, have shown no significant correlations between the activities of the glucosidases and soil pH (Dick et al., 1983; Eivazi and Tabatabai, 1990). This can occur due to the interaction of other intrinsic soil properties that affect enzyme persistence and expression (Acosta-Martínez and Tabatabai, 2000). Only when these are held constant such as when an Iowa soil was limed to achieve soil pH ranging from 4.9 to 6.9, the effects of soil pH on enzyme activity can be clearly observed (Acosta-Martínez and Tabatabai, 2000).

Our study found that some of the enzyme activities were correlated with organic C content of the soils (i.e., α-galactosidase activity in the Inceptisols, $r = 0.37$, $P < 0.05$; acid phosphatase activity in the Inceptisols, $r = 0.60$, $P < 0.001$), and when correlations were not found, could be explained by differences in organic matter quality among the soils. Generally, enzyme activities are correlated to soil organic matter content because the latter plays a key role as a precursor for enzyme synthesis (increases soil microbial biomass, which is an enzyme source), and in enzyme physical stabilization (Tabatabai, 1994). The fact that α-galactosidase, β-glucosidase, and β-glucosaminidase activities were significantly intercorrelated (r values up to 0.71, $P < 0.001$) may suggest these enzymes have similar origin and persistence in soil (Bandick and Dick, 1999). This may explain the reason that acid phosphatase activity was not significantly correlated to α-galactosidase, β-glucosidase, and β-glucosaminidase activities. Perhaps this enzyme has a different origin in the type of soils studied compared to the other enzymes investigated.

5. Conclusion

Our study found differences in the enzyme activities of soils within the RGA watershed relative to values reported for other ecosystems that may be related to the edaphic characteristics, parent material, hydrological, and/or climatic conditions influencing the soils. The results showed significant effects of soil order and land use on the soil enzyme activities involved in C, N, P and

S cycling in the tropical watershed studied. Generally, there were higher enzyme activities in the Oxisols compared to Inceptisols. Among the enzyme activities, β-glucosaminidase, β-glucosidase and α-galactosidase activities, involved in simple C substrates degradation, were similar in the Ultisols and Inceptisols. Acid phosphatase and arylsulftase activities were similar in the Oxisols and Ultisols, which were higher than in the Inceptisols. Lower soil enzyme activities were found under agriculture compared to pasture. Acid phosphatase and arylsulfatase activities were similar under pasture and forest. The enzyme activities studied play important roles in key biochemical reactions of C, N, P, and S nutrient transformation in soil. Thus, the significant reductions in these soil enzyme activities under cultivation compared to pasture and forest ecosystems, and lower values under Inceptisols should be taken in consideration as indicators of soil quality and environmental impacts in the watershed. For example, pasture and forest land uses were able to increase the activities of β-glucosidase, β-glucosaminidase, and acid phosphatase as a group in the Inceptisols to levels comparable to the Oxisols and Ultisols.

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